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# Fused filament fabrication of scaffolds for tissue engineering; How realistic is shape-memory? A review

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# 8 Abstract

9 Since the invention of additive manufacturing (AM) in the 1980s, great advances are today 10 conceivable thanks to considerable evolution in recent years. Medicine, and particularly tissue 11 engineering (TE), have high expectations regarding AM, which allows the manufacturing of 12 complex personalized parts. Among existing techniques, fused filament fabrication (FFF) is 13 very promising in the biomedical field, due to its many advantages, particularly for specific 14 applications such as scaffolds for TE. This review, in interaction with biomedical, process and 15 material sciences, aims to help researchers understand the importance of process 16 parameterization (build orientation, raster angle, layer thickness, etc.) combined with an 17 appropriate material choice, to develop optimized scaffolds using FFF. This review also 18 reflects the state of existing advances and opens perspectives on the subject, especially with 19 the use of biodegradable and biocompatible shape-memory polymers, the principle of which 20 will be revisited and the few studies concerning shape-memory scaffolds will be gathered.

Keywords: Additive manufacturing (AM); fused filament fabrication (FFF); scaffolds; shape memory polymers (SMPs); 4D printing

# 23 Nomenclature

- ABS: acrylonitrile butadiene styrene
- 25 AM: additive manufacturing
- 26 EBM: electron beam melting
- 27 FFF: fused filament fabrication
- HA: hydroxyapatite
- HDPE: high-density polyethylene

- 30 HIPS: high-impact polystyrene
- 31 MSC: mesenchymal stem cell
- 32 PA: polyamide
- 33 PBS: phosphate-buffered saline
- 34 PCL: polycaprolactone
- 35 PDLLA: poly (D,L-lactic acid)
- 36 PE: polyethylene
- 37 PEEK: polyether ether ketone
- 38 PEG: poly (ethylene glycol)
- 39 PEI: polyetherimide
- 40 PELA: poly (D,L-lactic acid-*co*-ethylene glycol-*co*-D,L-lactic acid)
- 41 PGA: poly (glycolic acid)
- 42 PHAs: polyhydroxyalkanoates
- 43 PLA: poly (lactic acid)
- 44 PLGA: poly (lactic-*co*-glycolic acid)
- 45 PLLA: poly (L-lactic acid)
- 46 PMMA: poly (methyl methacrylate)
- 47 PP: polypropylene
- 48 PS: polystyrene
- 49 PU: polyurethane
- 50 PVA: poly (vinyl alcohol)
- 51 PVC: poly (vinyl chloride)
- 52 SLA: stereolithography
- 53 SLM: selective laser melting
- 54 SLS: selective laser sintering
- 55 SM: shape memory
- 56 SME: shape-memory effect
- 57 SMP: shape-memory polymer
- 58 TCP: tricalcium phosphate
- 59 TE: tissue engineering
- 60 TPU: thermoplastic polyurethane
- 61 UHMWPE: ultra-high-molecular-weight polyethylene
- 62 UTS: ultimate tensile strength

# 63 Introduction

64 Additive manufacturing (AM) is defined in the ISO/ASTM 52900 standard as "process of 65 joining materials to make parts from 3D model data, usually layer upon layer, as opposed to subtractive manufacturing and formative manufacturing methodologies" [1]. Several different 66 67 AM techniques are commercially available and share common advantages like the possibility 68 to design devices for small-scale manufacturing at a low price in contrast to usual processing 69 techniques such as injection molding, whose equipment costs (e.g. expensive molds for each 70 part) must be made cost-effective by large-scale production. AM is a time-saver for rapid 71 prototyping and for personalized applications, and it also limits raw material waste as 72 compared to subtractive manufacturing processes. Finally, AM allows the designing and 73 printing of very complex structures that are not always achievable with traditional processing 74 techniques [2].

75 With this in mind, AM appears very interesting for the biomedical field as each patient is 76 unique and should have access to products adapted to their needs. It is even truer in the 77 precise field of tissue engineering (TE) and regenerative medicine, which is one of the major 78 topics of research. Indeed, most tissues are able to recover from minor damages only. For 79 example, bones have natural means of renewal and strengthening through the joint action of 80 osteoclasts and osteoblasts [3]. However, their capacity of renewal is limited in the case of 81 severe damage and tissues require medical intervention to repair, regenerate and strengthen. 82 This could be done thanks to the development of scaffolds and progress in TE. Scaffolds are 83 meant to provide a structure for cells to attach, to proliferate and differentiate. Traditional 84 techniques for scaffold production include solvent casting [4,5], electrospinning [6,7], 85 particulate/salt leaching [4,8], supercritical gas foaming [9] and so on. Still, these techniques 86 can somehow be unpredictable in terms of porosity control, while thanks to AM, processing 87 parameters could be chosen in order to obtain precise and reproducible structures and 88 porosity.

Techniques of AM used in biomedical fields include selective laser sintering (SLS), selective laser melting (SLM), electron beam melting (EBM), fused filament fabrication (FFF) and stereolithography (SLA) [10]. They differ in the transformation of the raw material into a succession of layers. SLS, SLM, EBM and FFF partially or totally melt the raw material (powder, pellets, filament) while SLA cures a liquid resin [11]. FFF appears attractive since the equipment and its maintenance are quite inexpensive and FFF is an easy-to-use AM technique [12,13]. It also requires lower cost materials (e.g. thermoplastic filament) than mostof the other techniques.

97 Of all thermoplastic polymers, their potential to be printed and, then, to be implanted in the 98 human body are critical points. From the technical side, adequate rheological and thermo-99 physical properties are essential. Duty *et al.* developed a model to assess material 100 "printability" and identify adequate processing conditions [14]. From the biological side, 101 biocompatibility and bioactivity are obviously necessary characteristics. A synthetic scaffold 102 is also meant to be degraded simultaneously with the development of new tissues, without 103 releasing toxic compounds.

Finally, shape-memory polymers (SMPs) were recently introduced to avoid invasive surgery. Indeed, SMPs permit the production of small parts (small temporary shapes) to limit the size of the surgical incision and to facilitate their insertion into damaged tissues. The parts then deploy thanks to an intrinsic stimulus (e.g. temperature, moisture) after implantation and recover their permanent shape. This feature could be very interesting to produce scaffolds for regenerative medicine.

110 Many reviews gather pieces of information about the different 3D printing techniques for 111 biomedical applications [15–20], AM of shape-memory materials for biomedicine [18,21,22], 112 biomaterials for scaffolds produced with traditional techniques and the interactions between 113 scaffolds and cells [20,23–26] but to the best authors' knowledge, no review focuses on the peculiar FFF technique to produce biodegradable and shape-memory scaffolds for TE. For 114 115 these reasons, the aim of this work is to show that FFF has great potential for the production 116 of scaffolds for TE and permits to overcome the problems encountered with traditional 117 techniques. In addition, it enables to print SMPs (4D printing). Required material 118 characteristics for body implantation will first be summarized. Then, the FFF process, its 119 advantages and adequate settings for scaffolding will be discussed. The last section will 120 introduce SMPs to conclude with scaffold 4D printing.

# 121 I. Required polymer characteristics to manufacture scaffolds for TE

## 122 I.1. Biocompatibility

123 The most important characteristic of a polymer intended to be in contact with or implanted in 124 the human body is its biocompatibility. Initially synonymous with inert, that is, not inducing a 125 reaction from the organism, the term "biocompatible" has evolved. Now, biocompatibility

- 126 also includes the ability of the material to obtain a positive response from the organism with a
- 127 minimal inflammatory reaction. A wide range of biocompatible and/or natural polymers is
- 127 Infinitial inflational feaction. If while funge of blocompatible analog induction
- 128 used or is being investigated to be used in biomedical applications (Table 1).

Category	Polymer/Copolymer	Applications	Ref	
Polyesters	PLA		[27–29]	
	PGA			
	PLGA	Sutures, scaffolds for TE, drug delivery		
	PCL			
	PHAs			
	PU	Bioadhesives, dentistry, breast implants,		
Polyurethanes		orthopedics, cardiovascular devices, wound	[30]	
		dressing, tissue reconstruction, contraception		
Polyolefins	PE, HDPE, UHMWPE	Bone replacement, tubing, packaging films	[31–34]	
	PP	Sutures, mesh, orthopedics, syringes, catheters	[35,36]	
Polyamides	PA 12, PA 6, PA 66,	Bone scaffolds, tubing, catheters, surgical	[34]	
	PA 612	instruments, sutures		
Vinul polymore	PVC	Tubes, blood bags, disposable medical devices	[37]	
v myr porymers	PVA	Drug delivery, wound dressing	[38,39]	
	PS, HIPS	Tissue culture trays, petri dishes, test tubes,		
Sturana nalumara		catheter trays, heart pump trays, epidural trays,		
Styrene polymers		respiratory care equipment, syringe hubs,		
		suction canisters		
Acrylic polymers	РММА	Intraocular lens, dental and mandibular	[40,41]	
Acryne porymers		corrections, bone cement		
Polyaryletherketones	PEEK	Spinal, orthopedic and maxillofacial surgery	[42,43]	
Natural polymers	Collagen chitosen	Drug delivery, implants for TE (scaffolds, skin		
	alginate, cellulose	replacement, bone substitutes, artificial blood	[44-48]	
		vessels and valves), wound healing		

129 Table 1 Biocompatible polymers and copolymers and their current/potential biomedical applications

HDPE=high-density polyethylene; HIPS= high-impact polystyrene; PA=polyamide; PCL=polycaprolactone; PE=polyethylene; PEEK=polyether ether ketone; PGA=poly (glycolic acid); PHAs=polyhydroxyalkanoates; PLA=poly (lactic acid); PLGA=poly (lactic-coglycolic acid); PMMA=poly (methyl methacrylate); PP=polypropylene; PS=polystyrene; PU=polyurethane; PVA=poly (vinyl alcohol); PVC=poly (vinyl chloride); UHMWPE=ultra-high-molecular-weight polyethylene

130

However, for scaffolding, some of these polymers are more suitable than others and additional properties (e.g. bioactivity, biodegradability, mechanical properties) are needed to meet the TE requirements. Copolymers, polymer blends [29] and composites with bioactive components are often produced to obtain these properties. Bernard *et al.* published a comprehensive review regarding the biocompatibility of polymer-based materials for medical devices containing precious information about the standards and regulations for biological evaluation [49].

#### 138 I.2. Bioactivity

Bioactive materials stimulate the biological response of the body. Chemical (e.g. surface
hydrophilicity), physical (e.g. surface roughness, Young's modulus) improvements, or both
can generate bioactivity.

142 Calcium phosphate ceramics such as hydroxyapatite (HA) Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub> and tricalcium 143 phosphate in its  $\beta$  form ( $\beta$ -TCP) Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> are often used as bioactive fillers [4,31,32,50–53]. 144 HA is highly biocompatible, osteoconductive and is similar to biological apatite (main 145 constituent of enamel, dentin and bone) [54]. Marra et al. used formulations of HA and 146 PCL/PLGA to produce scaffolds and highlighted viable cells and formation of collagen 500 147 µm deep into the scaffold containing 10 wt.% of HA [8]. Jeong et al. produced PLA/HA (5 148 wt.% and 20 wt.%) scaffolds by electrospinning and revealed viable cells and continuous cell 149 proliferation for 21 days [6]. They concluded that their scaffolds were effective to grow pre-150 osteoblasts (for bone regeneration). B-TCP also demonstrates inherent biomimetic potential 151 and can be used as a bioactive filler in scaffolds for TE [55]. It has the advantage over HA of 152 being much more soluble in biological media [56]. However,  $\beta$ -TCP is less advantageous in 153 terms of mechanical properties and HA and  $\beta$ -TCP are sometimes combined to achieve better 154 flexural strength and fracture toughness with HA and higher biodegradability with  $\beta$ -TCP 155 [57]. HA and  $\beta$ -TCP have also proved to increase surface roughness and thus, cell attachment, 156 proliferation, differentiation and tissue mineralization [5,58]. Other fillers such as bioactive 157 glasses (silicate glasses and glass-ceramics) can induce strong bonding between hard and soft 158 tissues and are of particular interest for TE [59]. In Serra et al.'s study, surface roughness 159 increased from  $117.72 \pm 60.50$  nm for PLA to  $1003.89 \pm 228.45$  nm for PLA-glass (50 wt.%) 160 [60,61].

161 Altankov and Groth pointed out that mammalian cells' interaction with materials was better 162 when their surface was hydrophilic than in the case of non-wettable surfaces [62]. They 163 observed a high proliferation of fibroblasts on hydrophilic clean glass whereas on octadecyl 164 glass, which is hydrophobic, no cell growth occurred. Hydrophilicity can also be tuned by 165 choosing the right material composition. For instance, PLGA is known to be more hydrophilic 166 than other polyesters such as poly (L-lactic acid) (PLLA), and hydrophilicity of their blends 167 can be tuned by varying PLLA/PLGA ratios [63]. Kao et al. used polydopamine to coat PLA 168 scaffolds and found that it greatly increased their hydrophilicity. They observed reduced contact angles from  $130^{\circ}$  for the pure PLA scaffold to lower than  $51.9^{\circ}$  and  $0^{\circ}$  (totally 169

170 hydrophilic) for the coated ones with increasing dopamine content in the coating solution 171 (from 1 to 2 mg/mL in 10 mM Tris, pH 8.5) [64]. They showed that polydopamine helped to 172 promote the proliferation of human adipose-derived stem cells and increased collagen I, cell 173 cycle, and cell adhesion. Wang et al. used cold atmospheric plasma to functionalize the 174 surface of PLA scaffolds for bone regeneration and showed that this treatment improved the 175 surface of their samples both physically and chemically [65]. Indeed, the water contact angle 176 measured on the surface of the scaffolds decreased from  $70 \pm 2^{\circ}$  to  $24 \pm 2^{\circ}$  and the nano-scale 177 surface roughness increased from 1.2 nm to a maximum of 27.6 nm. As a result, it promoted 178 both osteoblast and mesenchymal stem cell (MSC) attachment and proliferation.

179 Certain properties such as surface roughness can also be adjusted by selecting suitable180 processing parameters. This will be discussed in a later section.

# 181 I.3. Biodegradability

Some polymers have the advantage of being biodegradable. Such polymers could be implanted in the body and be automatically eliminated after an adequate time, thus avoiding a second surgical intervention to remove the implant. Polyesters and natural polymers presented in Table 1 can biodegrade in certain conditions. PLA, PGA and PCL are biodegradable polyesters that are already well implanted in the biomedical field and formulations containing PLA, PGA and PCL have already been approved by US and European agencies [18,66–68].

188 Their biodegradation is dependent on several factors and can be tuned, for example, by 189 controlling their molecular weight [69]. In the particular case of PLA, chirality influences its 190 biodegradation rate. The presence of D and L/D forms are known to increase the rate of 191 degradation of PLA [70]. Actually, crystallinity, directly related to the chirality of PLA, has a 192 huge impact on degradation. Amorphous regions degrade faster than crystalline ones, so that 193 tuning the material crystallinity induces changes in its degradation kinetics [25,71]. Chen et al. mention that blending PLLA with poly (D,L-lactic acid) (PDLLA) can increase the 194 195 kinetics of degradation of the final compound thanks to the amorphous nature of PDLLA [72].

PLGA (a copolymer of PLA and PGA) is the most studied biodegradable polymer for biomedicine because its properties can easily be tuned for specific applications by choosing the right lactic/glycolic molar ratio [71]. To determine the rate of degradation and the different steps of degradation of PLGA (different formulations) scaffolds, Wu and Ding used a phosphate-buffered saline (PBS) solution at 37°C, in order to mimic the natural body environment [73]. They measured the properties of PLGA (85/15 and 75/25) and PDLLA 202 scaffolds at different times after immersion in the solution. Their results showed that the 203 PLGA 85/15 scaffold of higher molecular weight resisted degradation longer than the 75/25, 204 while the PDLLA scaffold stayed almost intact after 26 weeks. Peng et al. used PLGA to 205 adjust the degradation rates of PCL/PLGA scaffolds, as PCL has the advantage of being 206 flexible but has a slow degradation rate and quite low bioactivity [74]. They showed that the 207 addition of PLGA drastically increased the kinetics of degradation because of PLGA 208 hydrophilicity. According to them, the 0.5PCL/0.5PLGA scaffold presents the ideal 209 degradation rate so that degradation and cell growth, and tissue maturation match (alveolar 210 bone).

PUs are also materials of choice for biomedical implants and Santerre *et al.* devoted an entire
review to the subject of their biodegradation [75].

213 Finally, the architecture of a whole device influences its rate of degradation. Bosworth and 214 Downes summarized the two schools of thought regarding this aspect [76]. The first is that 215 porous structures have more specific surface area and degrade faster than dense structures, 216 because of the facility for water molecules to penetrate them. The second is that porous 217 structures permit better evacuation of acidic products, decreasing autocatalysis. Results from 218 their own study on degradation of PCL scaffolds in PBS at 37°C tend to support the first 219 assumption that higher surface area to volume ratio induces faster degradation. A process 220 allowing a good control of the porosity seems therefore appropriate to tailor the degradation 221 kinetics of biomedical devices.

# 222 II. Fused filament fabrication (FFF)

#### II.1. Principle

Each AM technique needs to start from the design of a 3D part using computer-aided design or imaging techniques such as magnetic resonance imaging, computed tomography scans, etc. The resulting file (.stl) is then loaded in a slicing software to transform the 3D designed part into a layered structure and to generate a file in "G-code", which is a common programming language to define a toolpath. The AM machine then reads this file, containing essential information like the layer thickness or the deposition angle, and manufactures the 3D part.

FFF is based on extrusion that consists of melting/plasticizing raw materials in an extrusion head moving in the x and y directions. The molten material is pushed out of a nozzle to form a filament, which is deposited on a build plate to participate in the formation of a layer, thanks to the movements of the extrusion head governed by the G-code file. Once the first layer is

completed, the build plate moves down in the z direction for a distance equal to the layer 234 235 thickness and the deposition of the second layer starts. This cycle is reproduced until the 236 complete fabrication of the part, which can be of very complex shape, as seen in Figure 1. 237 Although the raw material is usually a thermoplastic polymer or a composite with a 238 thermoplastic matrix (e.g. PLA and hydroxyapatite particles [58]) in the form of filament 239 spools (Figure 2), a few innovative AM machines now allow the use of pellets (Freeformer 240 from Arburg, Germany; Pam Series P from Pollen AM, France, David from Sculptify, USA, 241 etc.).



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243

*Figure 1 : Complex shapes manufactured with an Arburg Freeformer (FFF)* 



244 245

Figure 2 : Schematic FFF process

Post-treatments of the resulting parts are sometimes required. For instance, a post-treatment is necessary when the part is printed with support material (also called sacrificial material) that has to be removed (Figure 3). With FFF machines equipped with two extrusion heads, the support material is different from the material constituting the part. In certain cases, it can be dissolved in a specific solvent, such as water to dissolve PVA. With FFF machines equipped
with only one extrusion head, the support material, printed in the same material as the part,
has to be mechanically removed. To facilitate this step, support material structures are printed
with a lower density than the part.



254

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Figure 3 : Post-treatment of a part; support material removal

256 In some cases, post-treatments are required to decrease the surface roughness. For example, 257 Garg et al. exposed acrylonitrile butadiene styrene (ABS) FFF-printed parts (with different 258 orientations) to cold vapors of acetone and showed that this treatment was very efficient [77]. 259 For instance, they reached a surface roughness of 2.5 µm with chemical treatment compared 260 to 6.27 µm before treatment for the 15°-oriented part, with minimal variations in the 261 geometric accuracy of the part. The applications considered by the authors were obviously not 262 targeted for biomedical applications, since high surface roughness tends to promote cell 263 adhesion.

264 Although FFF is very easy to handle to produce complex parts, it also has several drawbacks. 265 Compared to parts produced with traditional polymer processes, FFF-printed parts tend to 266 have lower mechanical properties because of their internal structure consisting of a succession 267 of filaments and layers. The deposition angles induce anisotropy in the part, and the 268 cylindrical geometry of the filament limits contact between adjacent filaments, which tends to 269 create intrinsic porosity and thus, to weaken the mechanical properties of the part. 270 Additionally, the mechanical properties of an FFF-printed part depend on the adhesion 271 strength between adjacent filaments that is led by the process parameters. The deposition of 272 warm layers onto cold ones creates thermal stresses, promoting negative phenomena like 273 shrinkage and warping effects and the delamination of adjacent layers. That is why, the choice 274 of adequate printing parameters is a key point.

## 275 II.2. Optimization of the FFF process

#### 276 II.2.1. For dimensional accuracy

277 Polymers should have suitable rheological and viscoelastic properties to be well transformed 278 by FFF and to reach shape and dimension stability during the entire process, meaning that 279 shrinkage and warpage should be avoided or limited as far as possible [14]. Shrinkage and 280 warpage can usually be minimized by using amorphous materials [78] as crystallization 281 induces volume reduction and undesired effects [79], by controlling the temperature of the 282 manufacturing chamber, the nozzle and the bed, and/or by mixing the polymer with fillers. 283 Wu et al. minimized the warpage of PEEK samples by choosing the right chamber (130°C) 284 and nozzle (350°C) temperatures [80]. The warping deformation decreased from 1.93 mm to 285 0.65 mm by increasing the chamber temperature from 90°C to 130°C. They attributed the 286 warpage to uneven crystallization occurring during rapid cooling, causing high internal stress, 287 and they concluded that the chamber temperature had more influence on warpage than the 288 nozzle temperature. Spoerk et al. revealed that a chamber temperature of 55°C (compared to 289 25°C) permitted to decrease internal stress in PP-borosilicate parts [81]. Indeed, during 290 printing, the part is submitted to the chamber temperature for a certain time. This is equivalent 291 to annealing, which releases stress and gives the material more time to crystallize. As a result, 292 parts printed in warmer construction environments tend to be less subject to geometric 293 variations after printing. Wang et al. showed the absence of warping deformation when the 294 chamber temperature was equal to the glass transition temperature  $(T_g)$  of the polymer [82]. 295 However, a balance must be found to minimize warpage but still enable layer solidification. 296 That is why, even though the Tg of ABS is close to 104°C, the chamber temperature (or 297 envelope temperature) is often lower (e.g. 70°C [83]; 75°C [84]).

Fillers are often used to reduce shrinkage and to control warpage [85]. Kochesfahani studied different formulations of PLA and minerals (talc, calcium carbonate, mica, kaolin etc.) to be processed via FFF [86]. The results revealed that minerals reduced shrinkage, warpage and improved the thermal stability of PLA. In some cases, minerals permitted to anneal the parts without losing their original shape and to reach maximum crystallinity at a reasonable speed, thanks to a better nucleation.

#### 304 *II.2.2. For surface properties*

Regarding the surface aspect, in general, a small layer thickness induces a low surface roughness, and adjusting processing parameters such as the part orientation during its production can influence the well-known staircase effect [13]. Depending on the application, 308 a high or low surface roughness may be preferred and layer thickness, surface slopes, 309 deposition angles, types of support and filling methods were shown to have an impact on it. 310 Both experimental and simulation studies allow to adjust the processing parameters as a 311 function of the desired properties [87,88]. The nozzle temperature also has an impact on 312 surface roughness, as revealed in Chaidas et al.'s study [89]. They measured the surface 313 roughness (average mean surface roughness Ra, surface roughness depth Rz, total height of 314 the roughness profile Rt and arithmetic mean width of profile elements Rsm) of PLA samples 315 printed with different nozzle temperatures and found that the increase in the temperature 316 induced decreases in all the roughness parameters (Ra, Rz, Rt and Rsm). In a similar study, 317 Wang *et al.* explained that higher temperatures enabled higher molecular movement and thus, 318 better diffusion, and managed to model the surface roughness as a function of printing 319 parameters [90].

#### 320 II.2.3. For mechanical properties

Many parameters can have an impact on the mechanical properties of an FFF printed part.
Figure 4 illustrates certain parameters that can be set on the machine and some features
resulting from the machine settings.



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325 326

Figure 4 : a) Different build orientations; b) Tunable parameters; c) Raster width and air gap; d) Example of an FFFprinted scaffold and its printing settings

327 The FFF process is well known to create anisotropic mechanical properties in the three 328 directions of printed parts. Actually, Chacón et al. studied the effect of the build orientation 329 on the mechanical properties of PLA samples [91]. They built samples upright, on-edge and 330 flat on the platform, resulting in different mechanical performances. Flat and on-edge samples 331 showed the highest mechanical properties (tensile and flexural strengths and stiffness) and 332 ductile trans-layer failure. On the contrary, upright samples showed the lowest mechanical 333 properties (e.g. tensile strength 78% to 37% lower than on-edge and flat orientations with different printing parameters). Indeed, during tensile testing, the weakest inter-layer bonds are 334 335 solicited for upright samples, which showed brittle intra-layer failure. Rodríguez-Panes et al. 336 revealed that increasing the infill (i.e. smaller air gap) resulted in denser specimens with less 337 porosity and thus, higher mechanical strength [92]. Popescu et al. reviewed more than 49 338 papers about the influence of FFF parameters and their interactions on the mechanical 339 properties of various printed parts [93]. They concluded that the most significant changes 340 were governed by the air gap (mechanical properties enhanced with a reduced or even 341 negative air gap), the build orientation, the raster angle, the layer thickness (small layer 342 thickness increasing the mechanical strength) and the infill percentage.

343 From a thermal point of view, Faes et al. quantified the influence of the inter-layer cooling 344 time (i.e. the time between the deposition of two consecutive layers) on the mechanical 345 properties of ABS parts printed by FFF [94]. They found that a longer inter-layer cooling time 346 induces weaker bonding and thus, decreases the mechanical properties (Young's modulus, 347 ultimate tensile strength (UTS) and elongation at break) when the part is built in the upright 348 direction. For example, the UTS of the upright samples decreased from 20.5 MPa to 13.3 MPa 349 when the inter-layer cooling time increased from 3.59 s to 45.27 s. This was not observed for 350 the flat samples whose UTS remained constant (19 MPa) while the inter-layer cooling time 351 increased from 54.92 s to 282.71 s. Indeed, when parts are printed upright and tested in 352 tension, their inter-layer bonds are loaded, whereas for flat samples, the intra-layer bonds are 353 solicited (stronger than the inter-layer bonds). Process temperatures also have a great effect on 354 the mechanical properties of the printed parts. Shelton et al. proved that the chamber 355 temperature conditions the envelope temperature, which is defined as the ambient temperature 356 around the printed part [95]. They varied the envelope temperature from 110°C to 170°C and 357 determined a 20% higher UTS for the sample made of ULTEM<sup>™</sup> 9085 resin with an 358 envelope temperature of 170°C compared to the sample with an envelope temperature of 359 110°C. They explained this improvement of the UTS by an increased neck development at 360 higher temperature. Garzon-Hernandez et al. represent neck formation as follows in Figure 5

361 [96].



362

363<br/>364Figure 5 : Formation of a neck between filaments (1) surface contact; (2) neck growth; (3) neck growth and molecular<br/>diffusion at the interface. Reprinted with permission from Ref. [96], copyright (2020), Elsevier.

Many studies and reviews were found in literature about the influence of different parameters on the mechanical properties of various printed parts [91,93–95,97,98] and a few studies even developed methods to predict the mechanical properties of a part by knowing the parameters used [96].

369 To sum up, FFF seems to be a promising process to manufacture specific and personalized 370 objects for biomedical applications since it has many advantages and few drawbacks, which 371 can be limited by setting optimization. The main limitation to the use of FFF in the 372 biomedical field is the lack of existing pharmaceutical grade polymers with the appropriate 373 properties to be printed with this technique [99]. Indeed, entire studies and reviews focus on 374 the influence of FFF processing parameters on mechanical properties of printed parts, degree 375 of bonding, and many other properties, but biocompatible and biodegradable polymers (with 376 the exception of PLA) are rarely found in the literature (generally ABS, polyetherimide (PEI), 377 PEEK). However, even though these current materials do not have the same properties as 378 polymers used for biomedical applications, the influence of the above-mentioned parameters 379 should follow the same trends. Also, Popescu et al. point out that further research should 380 evaluate the mechanical performances of FFF parts for specific applications (e.g. scaffolds) 381 and not only test specimens [93].

# 382 III. FFF for scaffolding

## 383 III.1. Potentially adequate materials for scaffold printing (FFF)

A few articles evaluate the performance of scaffolds manufactured by FFF. Among these 384 works, PLA is often studied because it has the required characteristics to be used in 385 386 biomedical applications and in AM processing [100,101]. PLA has a melting temperature 387 close to 160°C [102] and shows good dimensional stability and good adhesion to build 388 platforms, meaning that it does not need to be printed on a heated build platform [92,103]. Its 389 printing temperature range is rather low and Shahriar et al. estimated it was 160°C-190°C 390 with a maximum value of bonding length at 167°C (measured with two adjacent filaments in 391 an oven) [102]. Nevertheless, it is not rare to find higher printing temperatures in literature 392 such as in Grasso et al.'s work, where PLA is printed at 215°C [104]. Drummer et al. studied 393 the suitability of PLA/TCP for FFF and concluded that this composite material could be used 394 to print scaffolds for TE [78]. Amorphous PCL is also commonly used because of its low T<sub>g</sub> 395 close to -60°C and its high thermal stability (decomposition temperature of 350°C compared 396 to 235°C-255°C for other aliphatic polyesters tested), making it easy to print without 397 degradation [105,106]. Most importantly, PCL has very suitable rheological and viscoelastic 398 properties, facilitating its extrusion and enhancing its processability [18]. PLGAs are 399 thermolabile, which means that processing at high temperature such as during FFF can 400 provoke thermal degradation [107], the formation of toxic component and unpredictable 401 behavior in the human body since the integrity of the material can be altered [108]. PLGAs 402 integrity is highly reliant on the residence time during processing [109] and depending on 403 their lactic/glycolic molar ratio (higher lactic content increasing thermo-oxidative stability 404 [110]), PLGAs might not always be well suited for FFF. Still, some research proved the 405 feasibility of using PLGAs for scaffolding via FFF [107,111].

406 Many other polymers should be tested (Table 1) to evaluate the whole potential of scaffolds407 manufactured by FFF.

## 408 III.2. Scaffold optimization

- 409 The aim of scaffolds is to provide space and a support structure for cells to infiltrate, attach,
- 410 proliferate (Figure 6) and produce new extracellular matrix.





412

Figure 6 : Representation of a 3D printed (FFF) scaffold for bone regeneration

413 To do so, they need to possess specific properties such as biocompatibility, bioactivity, and 414 biodegradability, as discussed earlier. Other properties, such as high enough mechanical 415 properties (rigidity, resistance to impact and to friction, etc.) matching those of the tissues 416 meant to be colonized, are essential to maintain the structure after implantation. Porosity 417 features are also of great importance for tissue ingrowth and vascularization. The effect of 418 different pore sizes has already been reviewed in detail [112] as well as materials for scaffold 419 production, required structure, and mechanical properties of human tissues [113]. Some of 420 these properties directly result from the materials used but some result from the structure of 421 the medical device (scaffold), sometimes induced by the processing technique. In the 422 particular case of FFF, various architectures can be obtained (examples given in Figure 7) and 423 induce different cell behaviors.





Figure 7 : Examples of scaffold architectures induced by different raster orientations

426 Zein et al. studied several different FFF printed scaffold architectures, compared their 427 properties and showed that they could be significantly impacted by the processing parameters 428 [106]. Indeed, all the scaffolds were printed with the same material, namely PCL, but 429 differences in their architecture were generated by variation in the filament width, porosity or 430 lay down pattern (raster orientations). These induced properties such as a compressive 431 stiffness varying from 4 MPa to 77 MPa, a yield strength from 0.4 MPa to 3.6 MPa and a 432 yield strain from 4% to 28%. With the same value of porosity, raster orientations of 433  $0^{\circ}/90^{\circ}/180^{\circ}$  and  $0^{\circ}/60^{\circ}/120^{\circ}$  induced differences in the mechanical properties of the scaffolds. 434 According to Ostrowska et al.'s study, repetitive 0°/60°/120° raster orientation globally leads 435 to a high elastic modulus and the highest shear storage modulus as compared to  $0^{\circ}/15^{\circ}/30^{\circ}$ , 436  $0^{\circ}/30^{\circ}/60^{\circ}$ ,  $0^{\circ}/45^{\circ}/90^{\circ}$ ,  $0^{\circ}/75^{\circ}/150^{\circ}$  and  $0^{\circ}/90^{\circ}/180^{\circ}$ , and is one of the most commonly used 437 orientations in the treatment of bone diseases [114]. Differences can be explained by the 438 number of filament connections between layers (higher for  $0^{\circ}/90^{\circ}/180^{\circ}$  and  $0^{\circ}/60^{\circ}/120^{\circ}$ ) and 439 pore shape. For instance, square shape  $(0^{\circ}/90^{\circ}/180^{\circ})$  orientation) is associated to a low shear 440 storage modulus. Ostrowska *et al.* took their research a step further by analyzing the effect of 441 their PCL scaffold architecture on cell seeding, proliferation and osteogenic differentiation. 442  $0^{\circ}/15^{\circ}/30^{\circ}$  structure presented a higher cell number after 8 hrs since the tortuous architecture 443 of this scaffold helped trap the cells and enabled better seeding. However, after 35 days, the 444 architecture 0°/60°/120° displayed the highest cell number. They found only slight variations 445 in the differentiation of MSC on their constructs.

446 In most studies, though, researchers adjust both the FFF parameters and the scaffold 447 composition to achieve optimal properties. Bruyas et al. made a very complete study on 448 processing parameters and scaffold composition to print PCL/β-TCP scaffolds [55]. For 449 instance, they varied the strut distance (air gap), that influenced porosity, and measured the 450 mechanical properties under compression of scaffolds of different compositions. They 451 concluded that porosity was mostly dependent on the strut distance (more porous with longer 452 strut distance), whereas the  $\beta$ -TCP amount had only little influence. They measured Young's 453 modulus values varying from 12 MPa (highest strut distance and highest  $\beta$ -TCP amount) to 454 188 MPa (lowest strut distance and highest  $\beta$ -TCP amount), and yield strength values from 455 0.7 MPa (highest strut distance and highest β-TCP amount) to 15.4 MPa (lowest strut distance 456 and lowest  $\beta$ -TCP amount). According to them, the amount of  $\beta$ -TCP does not influence the 457 Young modulus in the case of high porosity scaffolds while increasing the amount of  $\beta$ -TCP 458 increases the Young modulus in the case of low porosity scaffolds. Regarding the yield

459 strength, the strut distance has clearly more influence than the composition of the scaffolds. 460 Composition, however, had a major effect on other properties. Indeed,  $\beta$ -TCP greatly 461 increased the surface roughness of the scaffolds (178.3  $\pm$  67.6 nm, 645.7  $\pm$  84.7 nm, 1193.6  $\pm$ 462 97.6 nm, and 1837.6  $\pm$  317.6 nm for  $\beta$ -TCP ratios of 0%, 20%, 40%, and 60%, respectively). 463 They performed accelerated ageing (alkaline conditions) and demonstrated that increasing the 464 amount of  $\beta$ -TCP in the composites tremendously accelerated degradation (less than 20 hrs to 465 fully degrade the 60% filled composite as compared to <1% weight loss for pure PCL after 54 466 hrs). These differences in properties induced various cellular behaviors. After 11 days of the proliferation study, the number of cells was about 50% higher on the scaffolds that contained 467 468  $\beta$ -TCP.  $\beta$ -TCP also had a great impact on osteogenic differentiation and 40% and 60% 469 formulations revealed an increase in the alkaline phosphatase activity at day 11.

470 Many examples of scaffold compositions for TE produced by FFF with several different
471 processing parameters and scaffold behavior toward cells were found in the literature and
472 gathered in Table 2.

- 473 Table 2 : Non-exhaustive list of scaffolds manufactured for TE printed via FFF and their main characteristics (Tn=nozzle
- 474 *temperature, Tp=platform temperature, \emptyset=diameter)*

Materials	Process parameters Device main features	Applications	Main results	Ref	
PLA-based scaffolds					
PLA	Tn: 195°C Nozzle Ø: 500 μm Pore size: 350 μm Strut diameter: 700 μm Porosity: 40%	Scaffold for bone TE	The pore size and strut diameter measured were very close to the design values ( $350\pm80 \ \mu m$ and $700\pm120 \ \mu m$ , respectively) Elastic modulus: $183.62\pm22.85$ MPa and $210.29\pm19.20$ MPa after post- heating >115°C for 300 s	[115]	
PLA	Tn: 210°C Nozzle Ø: 400 μm Layer thickness: 200 μm Speed : 30 mm.s <sup>-1</sup> Pore size: 550-600 μm	Scaffold for bone regeneration	Pore size obtained (550 $\mu$ m in XY and 620 $\mu$ m in XZ) close to the designed pore size (650 $\mu$ m in XY and 600 $\mu$ m in XZ) thanks to FFF accuracy No significant degradation after 8 weeks in PBS at 37°C	[116]	
PLA and PLA/5%HA	Tn: 200°C Nozzle Ø: 400 µm Layer thickness: 200 µm Speed: 50 mm.s <sup>-1</sup> Infill: 100% Perimeters: 3 Top/bottom layers: 3	Scaffold for bone TE	Successful fabrication of a porous 3D maxillary sinus via FFF Effects of HA: Good dispersion, ↑ flexural modulus (~7%) HA content should be increased	[117]	

PLA and PLA/HA 50/50	Tn(first layer) : 190°C Tn(further layers) : 180°C Nozzle Ø : 900 $\mu$ m Speed : 30 mm.s <sup>-1</sup> Theoretical porosity: 50% Size: 12.7 × 12.7 × 25.4 mm <sup>3</sup>	Scaffold for bone regeneration	FFF enables the use of particles, even in high amounts Effects of HA: ↑ surface roughness, homogeneous dispersion, ↑ porosity (from 39% to 55%), ↑ available surface area, amorphous material, ↓ stiffness (239 MPa to 124 MPa) but still compatible with that of trabecular bone (129 MPa)	[58]
PLA and PLA/15%HA	Tn(PLA): 180°C Tn(PLA/HA): 210°C Nozzle Ø: 350 $\mu$ m Layer thickness: 200 $\mu$ m Speed: 30 mm.s <sup>-1</sup> Pore size: 700 $\mu$ m (design 800 $\mu$ m) + interconnectivity Porosity: 30%	Self-fitting implants for bone replacement	Obtained pore size of 700 µm close to that of the modeled pore size of 800 µm thanks to FFF Effect of HA: crack inhibition during compression-heating-compression cycles	[118]
PLA and PLA/Titanium (Ti) particles	Tn: 210°C Tp: 90°C Nozzle Ø: 400 μm Layer thickness: 200 μm Speed: 30 mm.s <sup>-1</sup> Pore size: 560-790 μm Porosity: 47%	Scaffold for bone TE	FFF permitted to obtain a porous scaffold with controlled porosity and without cracks or defects, matching the initial design ↑ compressive/tensile properties with 5 vol% and 10 vol% of Ti Compressive strengths and moduli in the ranges of 20–30 MPa and 5– 7 GPa (between those of human cancellous and cortical bones) Improved biocompatibility with Ti particles Higher surface roughness with Ti (↑ cell attachment + osteogenic differentiation)	[119]
	PLGA	A-based scaffold	S	
PLGA; comparison with PCL	Tn: 110°C Pressure: 670 kPa Nozzle Ø: 350 $\mu$ m No chamber = fabrication at room temperature Size: 10 × 10 × 5 mm <sup>3</sup> Pore size: 600 $\mu$ m + interconnectivity	Scaffold for bone TE	FFF was found to be a promising tool for scaffold fabrication (uniform pore size, regular layers throughout the scaffold thickness) Compressive modulus: $2.55 \pm 0.11$ MPa PLGA = safe scaffold material Bone generation but high degradation (no scaffold structure remained) PCL was judged more promising (longer degradation rate = better for large defects) even without carrying cells before insertion	[111]
PLGA and PLGA/collagen II	Tn: 170°C Nozzle Ø: 150 μm Cylinder: Ø 7.5 mm; height 2.5 mm Stacking angles in 4D (0°/90°/45°/135°) or 8D	Scaffolds for cartilage TE	FFF induced a decrease of PLGA <i>M</i> w of 40% PLGA scaffolds: interconnected macropores PLGA/collagen II scaffolds: sponge- like structure (50–150 µm pores +	[107]

	(0°/90°/45°/135°/22.5°/67.5°/ 112.5°/157.5°) Spacing between adjacent fibers in the same layer: 0.35 mm, 0.65 mm or 1.15 mm		interconnectivity) Mechanical strength comparable to that of native cartilage ↑ cell seeding ratio from 70% to 90% for higher fiber spacing Smaller fiber spacing induced accumulation of acidic degradation products = autocatalytic effect and further degradation	
	PCL	-based scaffolds		
PCL	Tn: 80°C-90°C Nozzle Ø: 400 μm Speed: 5 mm.s <sup>-1</sup> Number of layers: 4	Scaffolds for TE	The FFF technology used did not strongly affect the geometry of the samples Complete cell (fibroblasts) adhesion and colonization of the structure	[120]
PCL and PCL/β- TCP; comparison with collagen	Tn: 110°C Pressure: 500 kPa Line width: 500 $\mu$ m Size: 10 × 10 × 0.32 mm <sup>3</sup> Pore size: 250 $\mu$ m	Membrane for guided bone regeneration (dentistry)	FFF permitted the use of biomaterials, blends and addition of powders + greater structural stability than that of commercially available collagen membranes Higher mechanical properties of collagen when dry Higher elastic modulus of PCL (171 MPa) and PCL/ $\beta$ -TCP (213 MPa) when wet (collagen: 12 MPa) Similar tensile stresses when wet: ~ 5 MPa PCL/ $\beta$ -TCP membrane demonstrated better bone regeneration performance than the collagen membrane	[52]
PCL + chitosan hydrogel filling (hybrid scaffold)	Tn:130°C Pressure: 1.5-3.0 bar Nozzle Ø: 400 $\mu$ m Speed: 1-3 mm.s <sup>-1</sup> Pore size: 325.2 ± 26.3 $\mu$ m Porosity: 62.4 ± 0.23%	Scaffold for bone TE	FFF allowed the printing of a porous PCL scaffold with well-controlled open and interconnected pores that allowed for a successful hydrogel filling afterwards Compressive strength: 6.7 MPa Low degradation ratio of PCL Favorable microenvironment for cell growth and osteogenesis	[121]
PCL and PCL/HA15%	Lay-down pattern: 0°/60°/120° Size: 10 × 10 × 8 mm Porosity: 65%	Scaffold for bone TE	FFF-made scaffolds may have a load- bearing application compared to foams that have low strength and low stiffness Stiffness: 7-11 MPa Increased after <i>in vivo</i> implantation = growth of tissue and ECM Constant rate of cell proliferation Tissue sheets were observed No significant difference between PCL and PCL/HA	[122]

476 Several benefits of FFF for the production of scaffolds can be identified from all of these 477 studies; the main one being the fine control of the scaffold structure. The differences between 478 the modeled scaffolds and the printed ones reside in the polymer thermal expansion during 479 printing or shrinkage but this can be rectified by designing slightly wider pores, knowing the 480 polymer thermal behavior, or readjusting the printing parameters. Also, it has been 481 highlighted that the structures of the scaffolds obtained by FFF are more likely to enable load-482 bearing, as compared to traditional techniques that often lead to scaffolds with low strength 483 and stiffness [122]. Moreover, the ability to use a variety of materials, even those composed 484 of particles, offers promising new possibilities. Therefore, a well-set FFF process combined to a good choice of materials and bioactivity "boosters" provide very promising perspectives for 485 486 scaffolding in TE.

487 However, further disadvantages of the FFF technique also emerge from these studies. 488 Material degradation can occur at high temperature, when hydrolysis-sensitive polymers are 489 processed, which is most often the case, but this problem can be partially solved, by 490 thoroughly drying the materials before printing. In addition, low-temperature deposition 491 manufacturing techniques based on FFF can be used [123]. The main drawback that can be 492 deduced by looking at Table 2 is the limited resolution conditioned by the nozzle diameter. 493 Indeed, the smallest nozzle diameter of all the studies described was found to be  $150 \,\mu\text{m}$ . This 494 results in a deposited filament with a thickness equal to or greater than this value and, 495 therefore, scaffolds with rather low porosities (30% to 65%) compared to what can be 496 obtained with other techniques (e.g. porosity of 94% with electrospinning [124]). In addition, 497 the use of small diameter nozzles limits the ability to process materials that contain particles 498 or fibers as they can cause clogging.

499 Nevertheless, the FFF process offers many new possibilities, among which the use of shape-500 memory polymers for scaffolding seems to be of particular interest.

# 501 IV. Shape-memory polymers for scaffolding

# 502 IV.1. Principle of SMPs for biomedical applications

503 Shape-memory polymers (SMPs) are of great interest in biomedical applications based on the 504 principle that a small temporary shape can be used to avoid invasive surgery and facilitate 505 patients' healing (Figure 8).



506

517

518

507 Figure 8 : Example of the interest of using SMPs for minimally invasive scaffold implantation for large bone defects

508 SMPs can recover from a temporary shape to their permanent shape under certain external 509 stimuli, such as water [125], pH [126], light/laser [127–129], magnetism [130] or temperature 510 [131,132]. Most reported SMPs are thermo-activated and have two different phases, a hard 511 phase and a switching phase [133]. They can be deformed from their permanent shape (Figure 512 9 a) to a temporary shape by increasing temperature above their transition temperature ( $T_{trans}$ ) 513 (Figure 9 b) and by applying a certain strain (Figure 9 c) to respectively soften and deform the 514 switching segments (reversible). The temporary shape is then fixed by decreasing temperature 515 below T<sub>trans</sub> and maintaining the strain (Figure 9 d). By re-increasing temperature above T<sub>trans</sub>, 516 the deformed part recovers its permanent shape through the hard segments (Figure 9 e).





519  $T_{trans}$  is generally a melting temperature ( $T_m$ ) or a  $T_g$ , depending on the polymer type 520 (thermoset, thermoplastic, blend, copolymer, amorphous or semi-crystalline polymer) and on 521 the type of cross-links. Cross-links can be chemical (covalent bonds) and physical (weak 522 interactions, crystalline domains, entanglements, etc.), and enable to set the permanent shape 523 and to return to it upon heating above  $T_{trans}$  while the switching segments are soft [134].

524 The ability of an SMP to recover its permanent shape can be quantified by the shape-recovery 525 ratio  $(R_r)$  according to equation (1).

$$R_r = \frac{\varepsilon_u - \varepsilon_{p(N)}}{\varepsilon_m - \varepsilon_{p(N-1)}} \times 100 \tag{1}$$

526 Where N is the cycle number,  $\varepsilon_p$  is the strain of the stress-free sample before applying yield 527 stress.  $\varepsilon_u$  is the strain of the stress-free sample after the programming step and  $\varepsilon_m$  is the 528 maximum strain under loading [135]. The higher the  $R_r$ , the more complete the recovery of 529 the part.

530 Shape-fixity ratio ( $R_f$ ), on the other hand, characterizes the material's ability to keep its given 531 temporary shape. It can be calculated by following equation (2) [135].

$$R_f = \frac{\varepsilon_{u(N)}}{\varepsilon_m} \times 100 \tag{2}$$

Achieving a high  $R_f$  is essential to prevent the part from premature expansion and recoil [136]. Figure 10 represents these notions. After heating (1), the polymer is loaded (2) and cooled down under loading (3). Then, the scaffold is unloaded (4) and goes from its maximum strain under compression ( $\varepsilon_m$ ) to its strain without stress ( $\varepsilon_u$ ), and it can be noticed in this example that shape fixity is not equal to 100% as  $\varepsilon_m \neq \varepsilon_u$ . After being reheated (5), the scaffold recovers its permanent shape but does not reach a 0% strain, revealing that shape recovery is not optimal either.







541 Various chemical and physical networks can trigger shape-fixing and shape-recovery 542 mechanisms, dividing SMPs in several different categories. Liu et al. classify them in four categories: chemically cross-linked glassy thermosets, chemically cross-linked semi-543 544 crystalline rubbers, physically cross-linked thermoplastics and physically cross-linked block 545 copolymers [137]. Other classifications also exist. Sun et al. classified SMPs into two 546 categories: covalently/physically cross-linked polymer network structures and polymer blends 547 [138]. Indeed, blending two immiscible polymers together permits to generate two immiscible 548 phases, with one of the polymers being highly crystalline and acting as the hard phase while 549 the other one is amorphous and acts as the switching phase. Karger-Kocsis and Kéki 550 distinguish thermo-activated SMPs as a function of their T<sub>trans</sub>, which is either a T<sub>g</sub> or a T<sub>m</sub> 551 [139].

552 SMPs are used in biomedical applications based on the facts that the body has a temperature 553 of 37°C (for heat-activated SMPs), that its pH is about 7.4 (for pH-activated SMPs) and that it 554 contains water (for water-activated SMPs). Sometimes, SMPs with several stimuli can be 555 obtained, such as in the study of Sessini *et al.*, who designed blends of ethylene-vinyl acetate 556 and thermoplastic starch responsive to both humidity and thermal stimuli [140].

#### 557 IV.2. Biodegradable and biocompatible SMPs

558 PUs include thermosetting PUs and thermoplastic PUs, also known as TPUs. The latter are 559 copolymers with alternating hard and soft segments. Depending on the nature of these 560 segments, their proportion, etc., an impressive variety of TPUs can be synthesized with all 561 kinds of properties, such as shape memory (SM) for some of them. Biodegradable PU-based 562 SMPs seem to have potential for medical applications because of their properties such as their 563 biocompatibility and their T<sub>g</sub> that can be tailored [141]. PCL, PLLA, PLGA, and many others, 564 can be used as soft segments [142]. However, elasticity is not always optimal and the rate of 565 biodegradation of such PU-based SMPs is lower (weight loss < 2.5% after 10 weeks of 566 immersion in PBS at 37°C) than that of other common biodegradable polymers (weight loss 567 close to 65% after 10 weeks for PLGA in the same conditions [63]). Blends of TPU and other 568 polymers such as PLA are commonly used to enhance the degradation rate of the resulting 569 material and as a counterpart, increase the other polymer SM properties [143,144]. Many 570 other biodegradable polymers (PLA, PCL etc.) present SM properties. However, the main 571 problems are that the recovery stress, shape-recovery, shape-fixity ratios, etc. may not be high 572 enough to be used for very precise applications such as in the biomedical field. Moreover, 573 PLA is brittle, poorly resistant to impact and has a low elongation at break, reducing its 574 suitability as SMP with high strain ratios. For these reasons, polymers are rarely used pure, 575 but rather blended with other matrices, additives and/or plasticizers. Custom copolymers can 576 also be synthesized to meet specific needs. In Zheng *et al.*'s research, the addition of particles 577 (HA) improved the shape-memory effect (SME) of PDLLA composites, which exhibited 578 higher shape-recovery ratios (over 95%) as well as faster recovery times (higher speeds of 579 recovery) as compared to pure PDLLA ( $R_r$  around 80%) [145]. Zhang et al. managed to 580 enhance PLA elongation at break by blending it with biodegradable polyamide elastomer, 581 while providing it with remarkable SM properties (recovery to permanent shape after 100% 582 strain in 8 s and 3 s at 80°C and 90°C, respectively) [146]. However, in order to be used in the 583 biomedical field, the T<sub>trans</sub> of SMPs should be lower than 60°C and this temperature should 584 only be maintained for a short period of time to avoid tissue necrosis [147]. The use of 585 plasticizers can be a good way to decrease the T<sub>trans</sub> of SMPs by enhancing chain mobility, 586 while increasing the elongation at break of fragile polymers such as PLA. Poly (ethylene 587 glycol) (PEG) is often used because of its biocompatibility and has been found in numerous 588 studies, blended (or copolymerized) with PLA and other polymers [148–151]. Cai et al. were 589 able to decrease the T<sub>trans</sub> of PLA/TPU blends and noticed a threshold value (10% PEG) above

which the increase in SM properties was no longer significant [150]. In the study of Guo *et al.*, PEG blended with PLA permitted to obtain suitable morphology (fewer pores, as samples were produced by solvent evaporation), hydrophilicity, and mechanical properties without too much impacting the SM properties [149]. Peterson *et al.* published a complete review dedicated to biodegradable SMPs in medicine, where a table containing 50 different formulations can be found [152].

## 596 IV.3. 4D printing for scaffold production via FFF

4D polymer printing combines the advantages of AM and those of SMPs to make smart objects. With time as an additional dimension to 3D printing, the term 4D printing was introduced. An example of a 4D printed object is given in Figure 11. A heart-shaped part was 4D printed via FFF and placed in hot water ( $\sim$ 70°C) to be deformed into a temporary shape (Figure 11-0 s) by holding the part during cooling. The temporary shape was then placed in a hot water bath at 70°C and its recovery was recorded until the part regained its permanent shape (Figure 11-1 min 30 s).



604

605 Figure 11 : 4D printed heart-shaped object taking back its permanent shape from a temporary shape (0 s) in hot water

606

~70°C; TPU with a  $T_{trans}$  of  $43^{\circ}C$ 

As mentioned earlier, not all thermoplastics are easily printable via FFF and are well suited to biomedical applications and many fewer have SM properties. Therefore, only a few 4D printed (FFF) scaffolds were found in the literature. Senatov *et al.* used PLA to manufacture SM porous scaffolds (Figure 12 and Figure 13) because of its suitable rheological and SM properties, due to physical entanglements of the long chains of PLA, playing the role of fixing phase [118,153].



614 *Figure 12 : 4D printed scaffold programming and recovery. Reprinted with permission from Ref. [118], copyright (2016),* 615 *Elsevier.* 

613

616 They added inorganic particles of high stiffness, in this particular case, HA, to provide 617 bioactivity as well as an additional fixing phase. In their first study, they almost doubled the 618 compression strength (at 15% strain) of the HA-filled scaffold as compared to that of the pure 619 PLA one and achieved a great shape-recovery ratio of 98% after the first compression cycle 620 [118]. They also reached a maximum recovery stress of 3 MPa at 70°C but concluded that the 621 SME activation temperature should be decreased for their scaffolds to be deployed in the 622 human body. In the second study, they revealed that their PLA/HA SM scaffolds induced 623 viable MSCs and stimulated their proliferation [153].



Figure 13 : PLA/HA 4D printed (FFF) scaffold showing, in green, MSC colonization at its surface. Reprinted with permission from Ref. [153], copyright (2017), Elsevier.

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625 626

627 Kutikov et al. studied the SM properties of thermoplastic amphiphilic poly (D,L-lactic acid-628 co-ethylene glycol-co-D,L-lactic acid) (PELA) and HA-PELA composites and successfully 629 printed (via FFF) biodegradable scaffolds from these materials, which exhibited rapid 630 recovery (~3 s) at 50°C [147]. The SME of amphiphilic polymers can be attributed to segregation between the hydrophilic and hydrophobic phases, where the phase with the 631 632 highest thermal transition induces the hard segments while the other phase constitutes the soft part. Hendrikson et al. used a shape-memory PU DiAPLEX® MM 3520 from SMP 633 634 Technologies Inc. to print scaffolds and found maximum recovery at 37°C after 30 min [154]. They tried two raster orientations,  $0^{\circ}/45^{\circ}/90^{\circ}$  and  $0^{\circ}/90^{\circ}/180^{\circ}$  and revealed that the scaffolds 635 636 built with the  $0^{\circ}/45^{\circ}/90^{\circ}$  raster orientation exhibited a higher shape recovery. As the surface 637 of the scaffolds was not adequate for cell attachment, they functionalized them by covalently 638 binding type I collagen. Results of MSC culture at 37°C for 14 days revealed 100% cell 639 viability, leading to the conclusion that these scaffolds have potential to be used for tissue 640 regeneration. However, the scaffold biodegradability was not assessed.

4D printing of scaffolds via FFF encounters similar difficulties as 3D printing via the same
printing technique (limited mechanical properties, anisotropy, shrinkage/warpage of the parts,
thermal degradation, rather low scaffold porosity, impossibility to use polymers other than
thermoplastics). In 4D printing by FFF, these difficulties are more apparent as the choice of

645 materials is even more limited, particularly for the production of scaffolds. Indeed, these 646 materials must be biocompatible, biodegradable, possess SM properties (with  $37^{\circ}C < T_{trans} <<$ 647 60°C), in addition to being thermoplastic. These materials are rare, as evidenced by the 648 scarcity of studies on the subject, and the development of new polymers meeting these criteria 649 is growing.

# 650 Conclusions and perspectives

Additive manufacturing and especially fused filament fabrication (FFF) have a great future for medical applications, and affordable implants adapted to the patients' needs should become more common in the biomedical and regenerative medicine fields.

654 Many advantages of the FFF processing technique were highlighted in the reviewed studies, 655 such as the possibility of printing complex load-bearing parts like scaffolds for tissue 656 engineering (TE). Studies revealed the possibility of finely controlling the properties of the 657 scaffolds (biocompatibility, bioactivity, biodegradation, mechanical properties, shape 658 memory, etc.) by choosing appropriate materials (neat polymers, filled polymers, copolymers, 659 polymer blends, etc.) and suitable scaffold structures (depending on process parameters like 660 raster width, air gap, layer thickness, angle deposition, etc.). On the one hand, results 661 described in this review are very promising and tend to encourage researchers to continue to 662 optimize FFF printed scaffolds. On the other hand, a severe lack of available biomaterials 663 well suited for scaffolding and developed especially for FFF was pointed out, opening up new 664 research opportunities.

665 Moreover, many studies deal with the influence of printing parameters on the properties of the 666 parts but these studies generally use materials that are not adapted for scaffolding. Research 667 also seems to be at an academic level since the printed parts are often test specimens and not 668 parts for special applications. This induces many research perspectives in the field of 669 scaffolds for TE produced by FFF, since printing parameters have an influence on their 670 properties. This has been addressed in a few studies but this has rarely been combined with 671 shape-memory polymers (SMPs), even though printing parameters seem to have a great effect 672 on their efficient deployment in the human body. Nevertheless, the few studies concerning 4D 673 printing for the production of scaffolds are very encouraging and make the use of SMPs 674 realistic for this kind of application.

# 675 Declaration of interest

676 The authors declare that they have no known competing financial interests or personal 677 relationships that could have appeared to influence the work reported in this paper.

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# Material's features

Biocompatibility Bioactivity Biodegradability

# FFF settings

Temperatures Layer thickness Deposition angles Filling rate Build orientation

# 3D printing (FFF) of scaffolds

# Scaffold properties

Pore size Porosity Mechanical properties Microstructure Shape-memory Behavior *in vivo* 

